Quality and quantity of genetic relatedness data affect the analysis of social structure

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Quality and quantity of genetic relatedness data affect the analysis of social structure

Vivienne Foroughirad[1] | Alexis L. Levengood[2] | Janet Mann[3] | Céline H. Frère[2]

[1]Division of Marine Science and Conservation, Duke University Marine Laboratory, Beaufort, North Carolina

[2]Global Change Ecology Research Group, University of the Sunshine Coast, Maroochydore DC, Queensland, Australia

[3]Departments of Biology and Psychology, Georgetown University, Washington, District of Columbia

Correspondence
Vivienne Foroughirad, Division of Marine Science and Conservation, Duke University Marine Laboratory, Beaufort, NC.
Email: vforoughirad@gmail.com

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Abstract

Kinship plays a fundamental role in the evolution of social systems and is considered a key driver of group living. To understand the role of kinship in the formation and maintenance of social bonds, accurate measures of genetic relatedness are critical. Genotype-by-sequencing technologies are rapidly advancing the accuracy and precision of genetic relatedness estimates for wild populations. The ability to assign kinship from genetic data varies depending on a species' or population's mating system and pattern of dispersal, and empirical data from longitudinal studies are crucial to validate these methods. We use data from a long-term behavioural study of a polygynandrous, bisexually philopatric marine mammal to measure accuracy and precision of parentage and genetic relatedness estimation against a known partial pedigree. We show that with moderate but obtainable sample sizes of approximately 4,235 SNPs and 272 individuals, highly accurate parentage assignments and genetic relatedness coefficients can be obtained. Additionally, we subsample our data to quantify how data availability affects relatedness estimation and kinship assignment. Lastly, we conduct a social network analysis to investigate the extent to which accuracy and precision of relatedness estimation improve statistical power to detect an effect of relatedness on social structure. Our results provide practical guidance for minimum sample sizes and sequencing depth for future studies, as well as thresholds for post hoc interpretation of previous analyses.

KEYWORDS
cetacean, genotype-by-sequencing, kinship, relatedness, SNPs, social network

1 | INTRODUCTION

Genetic data have given us invaluable insights into understanding social structure (Amos, Schlotterer, & Tautz, 1993; McCracken Gary & Bradbury Jack, 1981; Morin et al., 1994), kin selection and inclusive fitness (Burland, Barratt, Nichols, & Racey, 2001; Dickinson & Akre, 1998), and the heritability of phenotypes (Ritland, 2000). Estimating relatedness between individuals is a fundamental first step in investigating all these phenomena. The precision and accuracy of genetic information used in estimating relatedness is dependent on the type and number of markers used, the method of marker discovery, filtering processes, and the method of estimation, in addition to the naturally varying characteristics of the population and genome in question. Within behavioural ecology, genetic relatedness has typically been calculated using small sets of highly polymorphic markers such as microsatellites or amplified fragment length polymorphisms. Microsatellites are the most common marker employed to infer parentage and kinship in wild animal populations (Jones et al., 2010; Städele & Vigilant, 2016), favoured for their tendencies to be highly polyallelic and conserved between species (Sawaya, Lennon, Buschiazzo, Gemmell, & Minin, 2012).

As sequencing technology improves in cost and efficiency, genotyping-by-sequencing methods that can capture more abundant markers, such as single nucleotide polymorphisms (SNPs) are quickly rising in popularity (Narum, Buerkle, Davey, Miller, & Hohenlohe, 2013). Methods such as restriction site associated DNA sequencing (RAD-sequencing) (Davey & Blaxter, 2010) have allowed researchers to obtain large sets of genome-wide sequence data in the absence of known markers, greatly expanding the number of informative loci that can be quickly characterized for non-model organisms.

Comparisons of SNPs and microsatellites have demonstrated that even small numbers of SNPs perform as well or better than microsatellites in estimating kinship and parentage (Attard, Beheregaray, & Möller, 2018; Hauser, Baird, Hilborn, Seeb, & Seeb, 2011; Kaiser et al., 2017; Santure et al., 2010). In addition, some studies have shown evidence that large panels of SNPs (>10,000) can estimate genetic correlations (e.g., heritability; Bérénos, Ellis, Pilkington, & Pemberton, 2014) or genomic inbreeding and relatedness (Wang, 2016), better than multigenerational pedigrees. Traditional estimates of genetic relatedness from small sets of markers have typically performed well with respect to bias, i.e., relatedness values averaged within large sets of kin accurately reflect expected values. These estimates can be useful for comparing average relatedness values between groups, but small numbers of markers also result in high variance within each category. This results in overlapping ranges of relatedness estimates between categories, which prevents the assignment of individual pairs to a specific kinship category and the reconstruction of logical pedigrees (Taylor, 2015; Van Horn, Altmann, & Alberts, 2008) as well the ability to measure the proportion of different relationship classes within a population (Csilléry et al., 2006). Assigning a pair to a single kinship category becomes increasing more difficult with the distance of the relationship, for example cousin-level relationships and beyond can rarely be reliably delineated, and therefore we cannot know what role, if any, they play in shaping social structure or inclusive fitness.

These methodological advances should be complemented by empirical validation of the power and precision of relatedness estimation methods from species with different mating systems, dispersal patterns, and levels of inbreeding (Kopps, Kang, Sherwin, & Palsbøll, 2015; Pemberton, 2008). Such guidance is necessary for researchers to make informed decisions regarding research design tradeoffs in effort and cost, such as between sample collection and sequencing methods. While progress has been made to characterize error in the relatedness estimates themselves, usually in relation to pedigree-based expectations (Goudet, Kay, & Weir, 2018; Wang, 2016), there is still little information about how accuracy and precision of these estimates affect downstream analyses of kinship, social behaviour, and social bonding.

In this analysis, we use demographic and behavioural records from a long-term study of residential Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) in Shark Bay, Western Australia to investigate variance in relatedness estimation with differing quantities of data and carry these results forward to investigate their downstream effects on inference in social structure. This system comes with the advantages of a multigenerational observed maternal pedigree combined with three decades of social associations. The Shark Bay population is bisexually philopatric with neither sex dispersing from their natal range (Tsai & Mann, 2013), allowing researchers to continue to observe both sexes throughout their adult lives. Furthermore, their social system is characterized by relatively high fission-fusion dynamics without stable groups (Aureli et al., 2008), such that individuals can frequently encounter both male and female maternal and paternal kin as well as unrelated individuals within their home ranges, and so can choose from all kinship categories when selecting social associates. Previous studies have demonstrated that despite bisexual philopatry, mothers maintain much closer ties to daughters than sons (Tsai & Mann, 2013; Wallen, Krzyszczyk, & Mann, 2017) and relatedness is likely most important in social bonds between adult females (Frère, et al., 2010), while males may employ different social strategies dependent on alliance formation (Krützen, Sherwin, Connor, & Barre, 2003).

We use genotyping-by-sequencing to generate a moderately-sized panel of high-quality independent SNPs and conduct parentage assignment, which we validate against our observed multigenerational maternal pedigree and combine to produce expected relatedness coefficients. We then use a combination of simulations and subsampling of our data set to investigate, in detail, the relationship between the amount of genetic data (number of individuals and number of markers) and increased kinship resolution. We then carry the sets of relatedness estimates forward into a social network analysis investigating the impact of relatedness on social associations. These analyses serve to illustrate how power and effect size can vary between models constructed with different amounts of data, and has implications for the comparison of social organization between populations.

This study contributes to a growing body of literature aimed at understanding the power of SNP markers and genotyping-by-sequencing methods to assess kinship in wild mammals (Andrews et al., 2018; Attard et al., 2018). Further, this is to our knowledge the first study for any mammalian species to investigate how the accuracy and precision of such relatedness estimation affects inference in the analysis of social structure.

2 | MATERIALS AND METHODS

2.1 | Data collection

Data were obtained from a longitudinal study of Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) in the eastern gulf of Shark Bay, Western Australia. The population is resident and stable, with little evidence of migration and both sexes exhibiting natal philopatry (Tsai & Mann, 2013). Since 1984, a study area of about 300 km^2 has been regularly surveyed and demographic, behavioural, and ecological data collected on over 1,700 individuals. All individuals were identified through photographs using individual markings on the fin and body (Wursig & Wursig, 1977; Bichell, Krzyszczyk, Patterson Eric, & Mann, 2017). Birth years are known for most individuals born

since the 1980s and were determined either from observation of the individual as a calf or estimated from the degree of ventral to dorsal speckling (Krzyszczyk & Mann, 2012). Mother-offspring relationships were determined though observations of offspring nursing or swimming in the nursing access position (under the mother in contact with her abdomen) (Mann, Connor, Barre, & Heithaus, 2000). The average weaning age of offspring is over four years (Mann et al., 2000) so for each mother-offspring pair we had on average 19.1 days of observations used to confirm the relationship.

Social associations were determined during these surveys by grouping individuals using a 10 m chain rule (Smolker, Richards, Connor, & Pepper, 1992). GPS locations of the groups were recorded when the boat was within 50 m of the group.

Between 2013 and 2017, skin samples were obtained during boat-based surveys using a remote biopsy system (Krützen et al., 2002) in accordance with the University of the Sunshine Coast Animal Ethics Committee approval. Samples were collected opportunistically, but with preference for animals that could be matched to a catalogue in real time for inclusion in behavioural analyses. Tissue samples were stored in either dimethyl sulphoxide or an RNA-stabilizing buffer and DNA was extracted via isopropanol precipitation with the Qiagen Gentra Puregene Tissue kit. This study includes genetic data from 272 animals, 128 males and 151 females, which represent 44% of animals greater than two years of age encountered in the study site during the sampling period (Figure S1).

2.2 | Sequencing and SNP selection

DNA was sequenced using restriction-associated digest methods at Diversity Arrays Technology in Canberra, Australia using their proprietary DArTseq technology. DArTseq is a reduced representation sequencing approach, similar to RAD-sequencing. DNA was digested with *Pst*I and *Sph*I barcoded, multiplexed, and sequenced on an Illumina HiSeq 2500. Sequencing generated an average of 2.04 million 77 bp reads per sample. Single nucleotide polymorphisms (SNPs) were identified using a hybrid approach (Rochette & Catchen, 2017), in which loci were first assembled de novo, and then the resulting sequences matched up to the genome of the closest related species available. This approach helps separate out false-positive loci and allows for the positioning of loci to help remove variants in linkage disequilibrium, without compromising the consistency of genotype calls. This approach is widely applicable for species which lack a genome or if a closely related species has a genome of much higher quality. SNPs were called using the DArTsoftS pipeline (Cruz, Kilian, & Dierig, 2013; Kilian et al., 2012) and mapped to a 114.5× high-coverage *Tursiops truncatus* genome (NIST Tur_tru v1) using NCBI BLAST (Altschul, Gish, Miller, Myers, & Lipman, 1990). This resulted in 9,928 SNPs called with an average density of 30.8 reads per sample per locus, 86.6% of which mapped to the genome with an E-value below 2.4×10^{-6}. Seven samples were run in duplicate from which we calculated a mean genotyping error rate of 0.01895 (range: 0.005–0.047) per sample.

Single nucleotide polymorphisms were selected for analysis if they were typed in at least 95% of individuals, had an average read depth of at least 10 and a minor allele frequency (MAF) of at least 0.01 (Anderson et al., 2010; Goudet et al., 2018). SNPs were checked for Hardy-Weinberg equilibrium using the mid-p adjustment routine in PLINK 1.9 (Graffelman & Moreno, 2013) and any SNP that fell outside the 0.05 *p*-value cutoff was removed. To control for linkage disequilibrium, we treated our large scaffolds (n = 216; $\bar{x}$ = 9.8 Mbp) as independent and we used a sliding window filter with a window size of 50, a shift of five, and a variance inflation factor threshold of two, also implemented in PLINK 1.9 (Chang et al., 2015). We set a maximum heterozygosity filter of 0.6 to remove any potentially paralogous loci, and additionally removed five SNPs that segregated perfectly by sex and were assumed to be XY paralogs (Table S1). We checked for the presence of mitochondrial DNA in our sequences by blasting against a South Australian *Tursiops aduncus* mitochondrial genome (GenBank accession KF570335.1; Moura Andre et al., 2013) but no matches were returned within the default thresholds, so we assumed our reads were all from nuclear DNA. Lastly, we removed any SNP that had been mistyped between two or more pairs of duplicate samples. This resulted in a total of 4,235 SNPs, and we retained all samples that were typed at a minimum of 95% of these loci ($\bar{x} \pm SD$ 99.86 ± 0.004; n = 272). Observed heterozygosity and expected heterozygosity were calculated for these samples using the R package *hierfstat* (Goudet, 2005).

2.3 | Relatedness estimation

To choose an appropriate relatedness estimator for our data set, we first tested several estimators on a set of simulated genotypes that had characteristics similar to our data. We used the software COANCESTRY (Wang, 2011) to simulate 100 pairs of genotypes for dyads in each of five categories: (a) parent-offspring r = 0.5, (b) half-siblings r = 0.25, (c) half-avuncular (e.g., aunt-niece) r = 0.125, (d) half-cousins r = 0.0625 and (e) unrelated pairs r = 0. We selected these categories as representative of the kinship categories most likely to be encountered in a polygynandrous population. The simulated genotypes had the same number of SNPs and distribution of alleles frequencies as found in our real data, as well as the same amount of missing data. We set the genotyping error rate to be the same for all loci at 0.019 based on the error rate calculated from samples run in duplicate. We then compared six relatedness estimators, four moment-based estimators: Wang (2002), Lynch and Ritland (1999), Ritland (1996), and Queller and Goodnight (1989), and two maximum likelihood estimators, DyadML (Milligan, 2003) and TrioML (Wang, 2007).

We evaluated estimators by comparing the Pearson correlation coefficients between our estimated and expected relatedness values and calculating the root-mean-square error (RMSE) within and across kinship categories in the simulated data set.

Once we had determined which estimators performed well on the simulated data, we used those estimators to calculated genetic relatedness between all pairs of individuals in our real data also using COANCESTRY. We then tested the sensitivity of those estimators to the

sample size of individuals and loci by resampling our real data and calculating population-wide variance in relatedness estimates. We compared results for seven smaller quantities of SNPs (50, 100, 200, 400, 800, 1,600, 3,200) and individuals (10, 20, 30, 40, 50, 100, 150) by randomly subsampling the data, recalculating allele frequencies and pairwise relatedness, and then computing the discrepancy between each pairwise relatedness value in the subsampled and full data set. We compared all variations of number of markers and number of individuals for a total of 64 combinations, running 100 iterations of each combination and reporting the median root mean-square error for each level of subsampling.

2.4 | Parentage assignment and pedigree reconstruction

We verified that these data could be used to recover our observed mother-offspring relationships by running a naïve parentage assignment using the R package *Sequoia* (Huisman, 2017). Sequoia uses a maximum-likelihood based approach to assign parentage based on the log-likelihood ratios of first, second, and third-degree over unrelated relationships. We set the Sequoia input parameters to our observed genotyping error rate and the default number of mismatches and minimum and threshold log-likelihood ratios. Sex and birth year were included for all sampled individuals. We included sibship clustering, which imputes predicted shared parents for sibling dyads in which the shared parent was not included in the data set. We used the full set of 4,235 SNPs and set the maximum number of offspring to 20 for sibship clustering, as females have only been observed to have a maximum of about seven offspring that survive past weaning over the course of the lifetime but the number of offspring a male could produce is unknown and possibly much greater.

2.5 | Pedigree and genetic relatedness comparison

We used our parentage assignments generated by *Sequoia* and our observed maternal pedigree to calculate expected pedigree relatedness values for a subset of pairs with known kinship status. We included all first-degree relatives (r = 0.5) but limited other relationships to only those pairs in which all four parents were known. We further required that third-degree and fourth-degree relative pairs (r = 0.125 and r = 0.0625) had to have at least three out of four parents genotyped in the sample, and all unrelated pairs had to have all four parents genotyped to be included. We used the R package *kinship2* (Sinnwell, Therneau, & Schaid, 2014) to calculate the expected pedigree relatedness coefficient. For the individuals that had only one known parent, we imputed unique second parents. We examined correlations and RMSE between pedigree and genetic relatedness for all pairs, and classification rates between pairs with expected pedigree relatedness values in the set (0, 0.0625, 0.125, 0.25, 0.5), excluding pairs with pedigree relationships intermediate to the categories above, e.g., r = 0.1875, for simplicity. We used our simulated data to set classification thresholds for the genetic relatedness estimates between the five kinship categories such that each category included the maximum amount of correct assignments (true positives) while limiting incorrect assignments (false positives) to <5% within each category. We then applied these genetic relatedness thresholds to our empirical data and compared classification rates that could be achieved from relatedness coefficients calculated from different number of markers (50, 100, 200, 400, 800, 1,600, 3,200). Allele frequencies were set to those calculated using all individuals in the study.

2.6 | Genetic relatedness and social network structure

Finally, we examined how the accuracy and precision of our relatedness measurements would affect inference about the relationship between relatedness and social associations in our population. To do this we modelled association rates between pairs of individuals using network-based regressions that included their genetic relatedness, age difference, and home range overlap. As the Shark Bay population is highly sex segregated (Galezo, Krzyszczyk, & Mann, 2018) and males and females are suspected to employ different strategies with respect to forming kin-based relationships (Frère et al., 2010; Krützen et al., 2003), we did not examine mixed-sex relationships and instead ran separate models for male-male and female-female pairs.

These models included data from a subset of 176 individuals out of the 272 total, 92 females and 84 males, who had at least 35 sightings post-weaning. Individuals in the population are typically weaned at around four years of age, but with a range of 2–8 years (Karniski et al., 2018; Mann et al., 2000). We selected an inclusion threshold of 35 sightings in order to accurately capture the degree of spatial overlap between individuals (see Supporting Information for details), as well as each individual's position in the social network (Stanton & Mann, 2012). We estimated the association rate between each pair of individuals using the simple ratio index (SRI; Cairns & Schwager, 1987) with a one-day sampling period (Equation 1).

$$\mathrm{SRI} = \frac{X}{X + Y_a + Y_b + Y_{ab}}$$

X is the number of days both individuals (A and B) were seen in the same group, Y_a is the number of days individual A was seen without B, Y_b is the number of times individual B was seen without A, and Y_{ab} is the number of days both individuals were seen but in separate groups. We selected the simple ratio index instead of the more commonly used half-weight index as the simple ratio performs more consistently on sampled data in the absence of information on true association rates (Hoppitt & Farine, 2018). For each pair, the index was only calculated over the time frame where both individuals were alive and post weaning. We censored availability by either death dates or six months after the last sighting date as death dates could not always be accurately estimated for individuals in our population. We modelled the response variable, association index, as a weighted binomial variable, taking the numerator

of the association index (number of days observed together) to be the number of successes and the denominator (total number of days either individual was observed) to be the number of trials (Whitehead & James, 2015). As network regressions perform poorly in the presence of missing data, we set the response for pairs that did not overlap in time (0.03% of pairs) to 0 with the number of trials (observation weights) equal to 1.

Age was included as the absolute difference in years between each pair. Home range overlap was measured as the volume of intersection between each pair's home ranges. Home ranges were estimated using kernel-based utilization distributions (UDs) implemented in *adehabitatHR* (Calenge, 2006). Utilization distributions were calculated using individually-specified reference bandwidth smoothing parameters (Worton, 1995), and a simplified boundary derived from the coastline was included as a barrier (Benhamou & Cornelis, 2010). Any remaining land area was removed from all final UDs and the probability densities of each UD were restandardized to 1 as in Strickland et al. (2017). Volume of intersection was then calculated for all pairs using the 90% kernel of the UD for each individual. Because this population is bisexually philopatric and lives in an overlapping mosaic of home ranges, we expected relatedness values to positively correlate with home range overlap. Therefore, we examined the correlation between these parameters and variation inflation factors before hypothesis testing.

We then ran a series of social network models, varying the number of individuals and the number of markers used to calculate their relatedness coefficients according to the subsampling scheme described above, and keeping the values for the response variable and the other predictors, home range overlap and age difference, fixed. We ran 100 iterations of each model with each of (20, 30, 40, 50, 60, 70, 80) individuals and (50, 100, 200, 400, 800, 1,600, 3,200, 4,235) SNPs. Allele frequencies were obtained from a subsample of individuals twice as large as those included in each model. For example, in the model with 20 female subjects, allele frequencies were calculated using 40 individuals, under the assumption that even when modelling a single sex, samples from both sexes would have been collected. We implemented each model as a logistic regression, with model intercepts and coefficients estimated using the regular generalized linear model routine in R, and then assessed parameter significance using the multiple regression quadratic assignment procedure (MRQAP). MRQAPs are a method of hypothesis testing specifically developed for nonindependent network data. MRQAPs use matrix permutations designed to reduce type I error that can result from the inherent autocorrelation of network data, which violates traditional regression assumptions (Krackhardt, 1988). We used the Dekker double semi-partialling method (Dekker, Krackhardt, & Snijders, 2007) as implemented in the R package *sna* (Butts, 2008), using the *netlogit* function modified to accept a weights parameter (number of trials). We chose the Dekker double semi-partialling method to minimize the correlation between the variable of interest and the control variables under permutation. We used the *z*-value pivotal test statistic and 1,000 permutations to assess significance.

To compare the effect size of relatedness between the data sets with different sample sizes of individuals and different numbers of markers, we used the generalized partial coefficient of determination (R^2_V) to measure the proportion of the variance in association strength that is predictable from the relatedness coefficient, implemented in the R package *rsq* (Zhang, 2017). We note that the calculation of effect sizes and power for network-based regressions are poorly understood, and therefore caution that the results presented should only be interpreted as relative rather than absolute measures of power.

Lastly, we compared the prevalence of type II error, i.e., falsely accepting the null hypothesis that relatedness has no effect on social association, in the subsampled models using alpha = 0.05 to assess significance throughout. All analyses were performed in R environment version 3.5.1 (R Core Team, 2018) unless otherwise noted.

3 | RESULTS

3.1 | Genetic relatedness estimation

Our full dataset included 272 individuals typed at 4,235 SNPs with a mean minor allele frequency of 0.17 and an observed heterozygosity (H_o = 0.241 ± 0.165) slightly less than expected (H_e = 0.243 ± 0.167). When we compared relatedness estimators on simulated data, we found that the maximum likelihood estimators consistently outperformed the moment-based estimators with respect to correlation between expected and observed relatedness, and RMSE within and across kinship categories (Figure S2; Table S2), with the exception of the LynchRd estimator which performed best for the half-cousin (r = 0.0625) category. Overall, however, all estimators gave comparable estimates on the simulated data set with correlation coefficients above 0.99 and all RMSE below 0.04. As the dyadic (DyadML) and triadic (TrioML) maximum likelihood estimators performed almost identically on the simulated data, we chose the DyadML estimator for subsequent analyses based on its computational speed.

When we applied the estimators to our real data, population-wide estimates of relatedness among the full and subsampled data sets showed obvious differences in the degree to which estimates improved with additional data (Figure 1). The maximum likelihood estimator had a third of the error of the non-likelihood estimators at the smallest sample sizes, but including more individuals actually increased population-wide error when low numbers of markers were used. Since the best estimates on simulated data compared to expected values produced RMSE values at or below 0.03, we took 0.03 as an arbitrary threshold to assess convergence relative to the full data set. Using the maximum likelihood estimator, this level of convergence was achieved with as little as 800 SNPs for any number of individuals. In comparison the QuellerGt reached the same threshold with 3,200 SNPs and 40 individuals, or 1,600 SNPs and 100 individuals. The average relatedness calculated using the dyadic maximum likelihood estimator for all 272 individuals sampled was 0.014 ± 0.045 (mean ± *SD*) and ranged from 0 to 0.57.

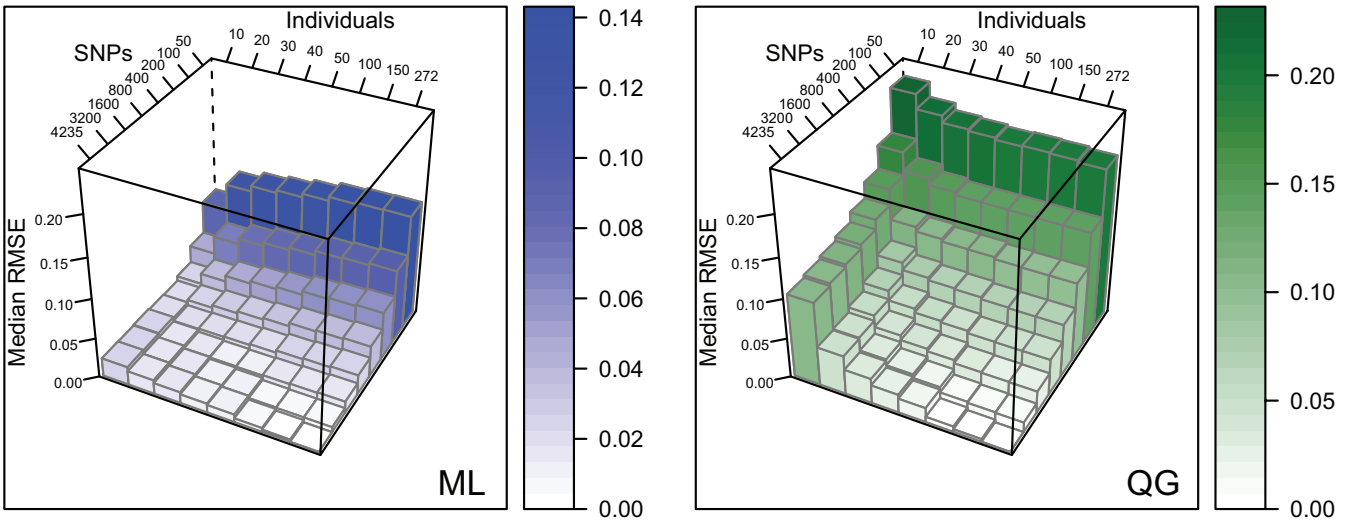

FIGURE 1 Variance in population-wide relatedness estimates when varying numbers of individuals and SNPs are used. Median RMSE is the median of the root mean square error relative to the full data set over 100 randomized subsamples at each level of data. The estimators shown are ML = dyadic maximum likelihood (Milligan, 2003) on the left in blue and QG = QuellerGt (Queller & Goodnight, 1989) shown on the right in green

3.2 | Parentage assignment and pedigree reconstruction

Of the 81 known mother-offspring pairs in our data set, 98.8% were correctly genetically assigned to their observed mother, with one individual not assigned a parent although the mother was in the sample. Six additional pairs were assigned in which the mother was not observed in the years immediately following the putative offspring's birth, but in no case was a mother genetically assigned that conflicted with the behavioural observation. Estimated mean confidence probabilities in the assignment calculated using Sequoia's *EstConf* function and 20 simulated pedigrees with 60% missing parents were 0.9696 for dams and 0.9722 for sires. Altogether, 87 mother-offspring and 53 father-offspring pairs were assigned, with an additional four mother-offspring and 23 father-offspring pairs imputed from the sibship clustering. We detected three pairs of full siblings in the data set, but the majority of sibling pairs (n = 129) were half-siblings. When we added these data to our known partial pedigree and calculated expected related coefficients for pairs with known kinship, we had 149 pairs at r = 0.5 level, 129 pairs at r = 0.25, 48 at r = 0.125, 20 at r = 0.0625, and 492 at r = 0, or unrelated (Figure 2).

3.3 | Pedigree and genomic relatedness comparison

In our simulated data set, we were able to use our estimates of genetic relatedness to discriminate between all five kinship categories by achieving true positive classification rates of 85%–100% for all categories while limiting false positives to <5% (Figure S3). When we applied the classification thresholds derived from simulated data to the empirical data, using all available markers there was no misassignment between pairs related at the r = 0.5, 0.25, and 0 levels. However even in our full data set, there was some misassignment between half-cousin (0.0625) and half-avuncular level relatedness (0.125), and half-avuncular and half-siblings (0.25) (Figure 3). We found we could attain 95% correct classification for r = 0, 0.25, 0.5, and 80% correct classification for the r = 0.125 category, but never more than 65% correct classification in the r = 0.0625 category with a high rate of false positives (Table 1). Some of the discrepancy between classification ability in the simulated and empirical data sets may stem from missing data in the pedigree, as there were few individuals for which more than one to two ancestral generations were known and genotyped.

When we applied the classification thresholds to our subsampled data sets, we found that the r = 0.5 category only required 50 SNPs for 80% correct classification and 200 SNPs for 95%, r = 0.25 required 400 SNPs for 80% correct classification and 1,600 for 95%, r = 0.125 required 3,200 SNPs for 80% correct classification but did not achieve 95% correct classification, and unrelated required 800 SNP for 80% correct classification, and 1,600 for 95% classification with our designated thresholds (Table 1).

3.4 | Power in social structure analysis

We examined how the variation in relatedness estimation affected the analysis of social structure by conducting a series of network regressions on subsampled data.

The Spearman rank correlation coefficient between home range overlap and pairwise relatedness in the full social data set (n = 176) was 0.26, and variation inflation factors were all less than two (Zuur, Ieno, & Elphick, 2010), so we incorporated both home range overlap and relatedness as predictors in the same models. We first ran two models using our full data set, one for male-male and one for female-female associations. For female-female pairs,

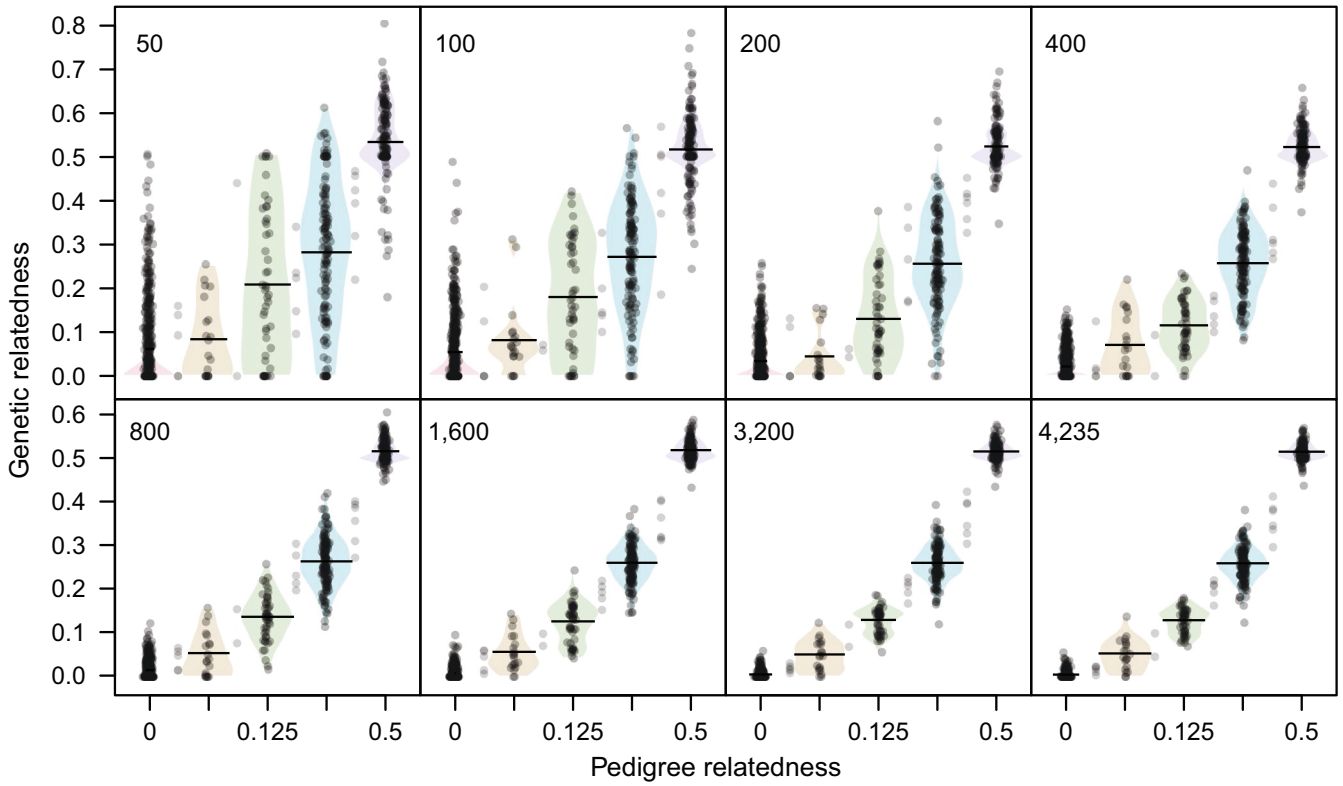

FIGURE 2 Distribution of genetic relatedness estimates calculated with different numbers of markers against expected relatedness values derived from the pedigree. Number of SNPs used is shown in the upper left-hand corner of each panel. Smoothed density curves are shown using shaded regions for the most common pedigree categories (0, 0.0625, 0.125, 0.25, 0.5), and pedigree values are slightly jittered for visualization purposes. Horizontal lines represent the mean for those categories

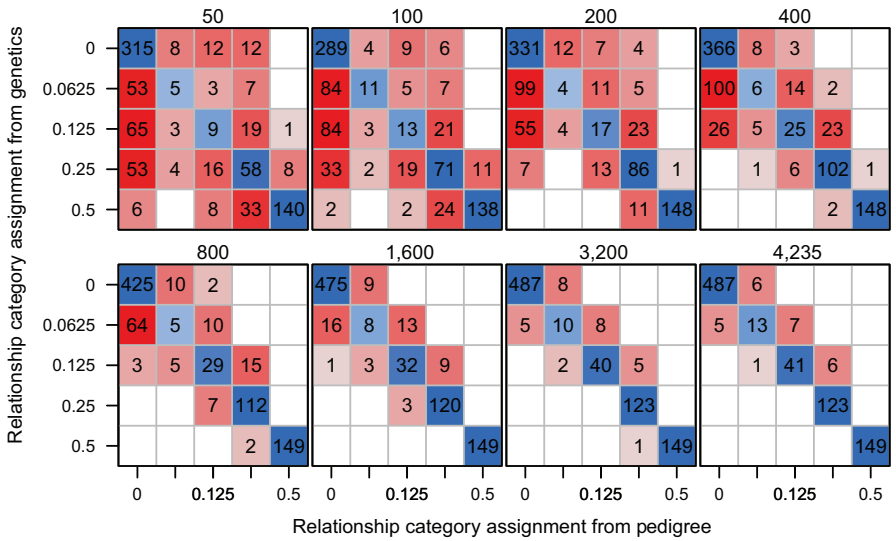

FIGURE 3 Confusion matrix of relatedness category assignments for five categories of pedigree relatedness. Correct assignments are shown along the diagonal in blue with incorrect assignment above and below in red, with shading proportional to the number of assignments. Number of pairs assigned to each category is reported within each square and the number of SNPs used is reported above each panel

both relatedness and home range overlap significantly predicted association rate, but age difference did not. We found that unlike for females, relatedness was not a significant predictor of association strength for male-male pairs in our model (Table 2). Therefore, we conducted our subsequent subsampling analysis using only female data.

TABLE 1 Pedigree versus genetic relatedness category assignment comparison. True positive rates are the proportion of pairs assigned in the same category using genetic data and the pedigree. False positive rates are the proportion assigned to each relatedness category using genetic data that do not belong in that category according to the pedigree

			Relationship category assignment									
			0		0.0625		0.125		0.25		0.5	
Number of SNPs	Correlation coefficient	RMSE	True Positive	False Positive	True Positive	False Positive	True Positive	False Positive	True Positive	False Positive	True Positive	False Positive
50	0.843	0.126	0.640	0.092	0.250	0.929	0.188	0.907	0.450	0.583	0.940	0.251
100	0.887	0.102	0.587	0.062	0.550	0.897	0.271	0.893	0.550	0.478	0.926	0.169
200	0.942	0.071	0.673	0.065	0.200	0.966	0.354	0.828	0.667	0.196	0.993	0.069
400	0.970	0.050	0.744	0.029	0.300	0.951	0.521	0.688	0.791	0.073	0.993	0.013
800	0.984	0.036	0.864	0.027	0.250	0.938	0.604	0.442	0.868	0.059	1.000	0.013
1,600	0.991	0.027	0.965	0.019	0.400	0.784	0.667	0.289	0.930	0.024	1.000	0.000
3,200	0.994	0.023	0.990	0.016	0.500	0.565	0.854	0.146	0.953	0.000	1.000	0.007
4,235	0.994	0.022	0.990	0.012	0.650	0.480	0.854	0.146	0.953	0.000	1.000	0.000

In the female model, relatedness was a significant parameter which explained about 12.8% of the variance as measured by partial R_V^2. We defined a type II error rate as the proportion of subsampled models in which the estimated effect of relatedness did not meet the threshold for significance even though it was a significant parameter in the full data set. We found that it took at least 60 individuals and 800 SNPs before a type II error rate less than 5% was achieved. Low numbers of SNPs resulted in higher rates of type II error and on average lower partial R_V^2 values, though with high variation (Figure 4). For models with smaller numbers of individuals, we found that the set of individuals chosen had a strong effect on the value of partial R_V^2, even when relatedness estimates were obtained from the full data set.

4 | DISCUSSION

In this study we investigated whether deriving genotypes via DArT sequencing could produce more precise estimates of kinship than those reported from studies using other genotyping methods which produce fewer markers for analysis. We then investigated whether those improved estimates provided increased power when modelling the effect of relatedness on social structure.

4.1 | Genotyping-by-sequencing and relatedness estimation

We demonstrate successful parentage assignment and good correspondence between pedigree and genomic relatedness using a moderately-sized panel of SNPs generated from DArTSeq. The correlation between pedigree and genomic relatedness that we achieved was comparable to that predicted by previous simulation-based studies using similar numbers of SNPs (Kopps et al., 2015).

Though we could not directly compare our results with those that would have been generated from the most commonly used type of marker, microsatellites, we can use values from the literature to generate a rough estimate. There are about 26 microsatellites that have been characterized for bottlenose dolphins with an average of about 6 alleles each in our population (Kopps, Kang, Sherwin, & Palsbøll, 2014). This suggests a scaling factor for translating informativeness from the number of microsatellites to number of SNPs to be within the 5–10 range reported in previous studies (Santure et al., 2010; Städele & Vigilant, 2016; Wang, 2016), and therefore the power we would get from the 26 known microsatellites would likely have been analogous to the results we obtained using about 200 SNPs.

We find that the correlation between relatedness estimates and expected pedigree relatedness improves up to about 1,600 independent SNP markers in a sample of at least 20 individuals. These estimates alone can also be used to develop classification thresholds that can distinguish between several kinship categories (parent-offspring, half-sibling, unrelated) with greater than 95% accuracy, and more distant categories (e.g., half-avuncular) with 80% accuracy. We note that the pedigree we used to assign expected relatedness coefficients was

TABLE 2 Model results from the logistic regression with multiple-regression quadratic assignment procedure for both male-male (n = 84) and female-female (n = 92) networks with 1,000 permutations each.

Model	Covariate	Coefficient	*SE*	*z*-value	*p*-value
Female-female	Intercept	−7.421	0.036	−205.191	–
	Relatedness	1.820	0.069	26.290	**<0.001**
	Home range overlap	6.585	0.051	129.342	**<0.001**
	Age difference	−0.012	0.001	−9.018	0.171
Male-male	Intercept	−7.727	0.042	−183.024	–
	Relatedness	0.278	0.161	1.723	0.658
	Home range overlap	7.778	0.052	148.793	**<0.001**
	Age difference	−0.078	0.002	−40.596	**<0.001**

Bold values denote statistical significance at the $p < 0.05$ level.

incomplete, and therefore some of the discrepancy between pedigree and genetic relatedness in our analysis may be inflated because of missing data. However, we also left out some relatively rare kinship categories (e.g., those with an expected r = 0.1875) and more distant (e.g., r = 0.03125) expected categories of kinship from our relatedness category assignment as they were either rare in our population, or rarely detected due to our shallow pedigree. Therefore, we had limited power to assess their accuracy and how they would affect the accuracy of other categorical assignments. The accuracy of our categorical assignments is only relative to the five kinship categories we selected for analysis, and applying these categorical thresholds to a whole population will result in misclassification for those excluded categories and higher rates of false positive assignments.

Maximum likelihood estimation of relatedness was the most robust estimator for our data over a range of sample sizes, but results suggest significant diminishing returns to prioritizing number of samples over number of markers. Relative error in some cases increased when a larger proportion of the population was sampled (Figure 1; Figure S2). Though somewhat counterintuitive, this result has been found in other studies (Kopps et al., 2015), and is a consequence of the error in estimating allele frequencies when the frequencies are calculated from small samples of individuals. For example, in the case of biallelic SNPs, if a dyad in a sample of 10 individuals shares a private allele that is identical by state, that minor allele's frequency is calculated as 0.20, which is considered when weighting the likelihood that the allele is actually identical by descent. Alternatively, if a dyad in a sample of 100 individuals shares a private allele that is identical by state, that minor allele's frequency is 0.02, which gives greater weight to the likelihood that the allele is identical by descent than 0.2 would. With large numbers of loci, a private or otherwise rare allele that is identical by state will have much less weight on the overall likelihood of coancestry, but with

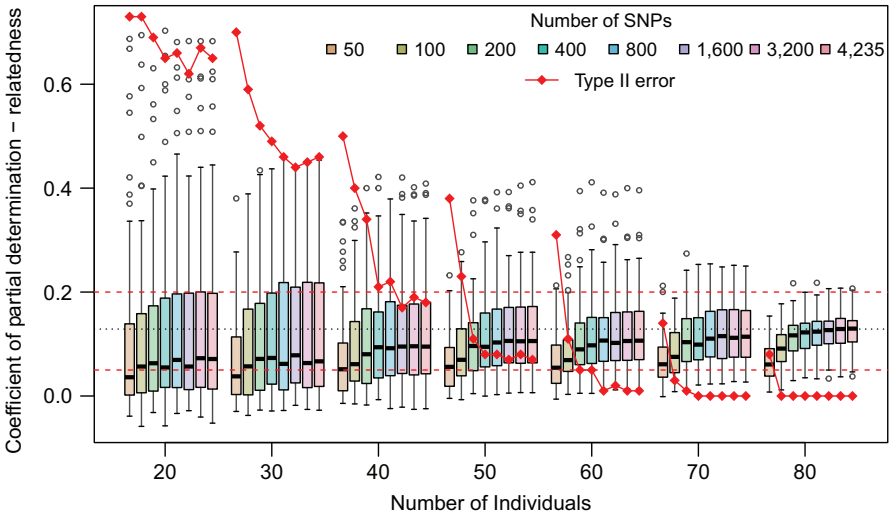

FIGURE 4 Box plots show the effect size of relatedness on association strength, measured as the coefficient of partial determination (R^2_V), across logistic network-based regressions with different levels of data. Box heights are the interquartile ranges (IQR) bisected by median values, and whiskers extend to 1.5 times the IQR. The black dotted line is the R^2_V value obtained from the full data set. The overlaying solid red lines show type II error rates, or the proportion of models in which the p-value obtained for the relatedness parameter is above the alpha = 0.05 threshold resulting in a type II error when the full data set is taken as truth. Red dashed lines show the 20% type II error rate (80% power) and 5% type II error rate (95% power)

small numbers of loci this will result in the tendency to overestimate relatedness, especially for pairs near the boundary condition (0 or unrelated). Since most pairs in the sample will be unrelated, this bias will outweigh any improvement for related pairs with rare alleles that are actually identical by descent. The method-of-moments estimators such as Queller and Goodnight's (1989) do not have a boundary condition at 0 and are statistically unbiased, but in many cases the increased variance in their estimates results in greater overall error than the maximum likelihood estimator. These effects may be somewhat remedied by choosing a higher minor allele frequency threshold, but this will result in fewer total loci included. Balancing these effects may depend on the precise research question, and in some cases false discovery rate procedures may also be appropriate (Skaug, Bérubé, & Palsbøll, 2010). Even with large amounts of SNP data, other parameters such as genotyping error, allelic dropout, and the true distribution of allele frequencies in the population may affect estimator accuracy and precision (Attard et al., 2018), and we recommend conducting simulations to choose the best estimator for each study.

As more assembled genomes become available, incorporating patterns of shared alleles along chromosomes (Browning & Browning, 2013) and the use of more sex-linked and mitochondrial markers may improve relatedness estimation, especially in discriminating among classes of relatedness that share the same expected coefficient, such as grandparents and half-siblings (both $r = 0.25$). Combining log-likelihood ratios with relatedness coefficients may also improve categorical discrimination in some cases (Städele & Vigilant, 2016), and new relatedness estimators are rapidly being proposed and evaluated (Goudet et al., 2018). Bayesian approaches that allow the incorporation of uncertainty into the relatedness estimates may also improve model inference (O'Hara, Cano, Ovaskainen, Teplitsky, & Alho, 2008). Importantly, much of our evaluative framework assumes that genetic relatedness is informative because it allows us to estimate a socially meaningful kinship category, rather than an absolute percentage of genome sharing (Speed & Balding, 2015; Wang, 2016). Depending on the method of kin recognition in each species, estimation of different aspects of relatedness may be more relevant.

4.2 | Relatedness estimation and power in social structure analysis

The results of our analysis suggest that male-male and female-female pairs experience different effects of relatedness on their association strengths. This is expected as several long-term studies of the population have shown that kin relationships are important for female bonding (Frère et al., 2010; Mann et al., 2012), while male-male bond formation has a more complicated relationship with kinship that varies with alliance structure (Krützen et al., 2003). We note that our results do not show that male kin do not form bonds, only that they do not do so at rates higher than expected based on their home range overlap.

In our social network models, we found that model precision (bias and variance in effect size estimates) consistently improved up to about 400 SNPs, with similar results for model power. In our data set, 80% power could be achieved with 40 individuals using relatedness estimates derived from 1,600 SNPs, or with 50 individuals using as little as 200 SNPs. After about 400 SNPs, model power and precision benefited much more from increasing the sample size than from increasing the resolution of the relatedness estimates.

Statistical assessment of the effect of relatedness on social associations in the wild will often suffer from low power for several reasons, including that network observations are by nature non-independent, and within the networks, kin can be relatively rare. The lack of independence in dyadic observations and potential structural autocorrelation can make them unsuitable for traditional ordinary least squares regressions because of high rates of type I error (Krackhardt, 1988). As a result, methods that correct for this bias, including MRQAPs, are conservative by design. Regarding questions involving kinship and relatedness, the highly skewed distribution of relatedness also means that there could be very different proportions of kin in small subsamples. In our sampled population about one out of every 16 dyads share a relatedness coefficient greater than or equal to $r = 0.0625$. Therefore, in models with 20 individuals (190 pairwise relationships), there are only 12 (95% CI: 6–19) or so pairs that can be classified as kin, and the actual proportion may vary widely based on whether the individuals are sampled randomly from the population, or in groups as they are encountered. This variation in the proportion of relatives due to sampling protocol could have significant effects on model output. Sex differences in kin affinity are also common in many populations and could affect the power and precision of network-based regressions if models are not split by sex. MRQAP methods cannot assess interaction effects, and including both sexes in a model in which there was an interaction effect would further reduce power.

In this analysis, we focused on a commonly used analytical framework in the interest of providing comparable and interpretable estimates of power rather than precise inference. While generalized linear models, and especially network extensions such as MRQAPs are a popular hypothesis testing framework for these types of analyses (Carter, Seddon, Frère, Carter, & Goldizen, 2013; Louis et al., 2018; Van Cise et al., 2017), the skewness inherent in the distribution of relatedness values and the potential for interactions between predictors may make this framework unsuitable for some types of social analyses. We note that with both moderate numbers of individuals (e.g., 30 of one sex, which may require sampling 60 individuals total) and markers (e.g., 200 SNPs, equivalent to about 30 microsatellites) we obtained a type II error rate greater than 50%. We suggest that some studies that use similar levels of data and find no effect of kinship on social associations employ caution when interpreting their results. Some studies which have reported no effect of relatedness on social structure are likely to be underpowered, especially if kin are important for only one sex or some life history stages. This is a potential explanation for why previous studies have been unable to find a generalized effect of relatedness on social structure between social and subsocial systems (Bouskila et al., 2015).

There are other types of models which may offer more power with less data, such as exponential random graph models (Silk &

Fisher, 2017), and structured randomization tests (Croft, Madden, Franks, & James, 2011), especially those that incorporate appropriate null models (Strickland et al., 2017; Farine, 2017). However, there has not yet been a comparison of these modelling approaches that has focused on inference about relatedness, especially when incorporating parameter resolution. Such a study would be very beneficial to questions about kin recognition, kin selection, and altruism in wild populations.

5 | CONCLUSIONS

Accurate measures of relatedness and how kinship affects the formation and maintenance of social bonds is important for understanding the adaptive value of sociality and the evolutionary drivers of group living (Silk, 2007). Making inferences based on kin selection and inclusive fitness theory (Hamilton, 1964) relies on knowing the relatedness between pairs of interacting individuals in order to properly weight the cost versus benefit equations. For long-lived, slow reproducing, and polygynandrous species with cryptic paternity, estimating relatedness from shared genetic markers may be the only way to obtain kinship information.

The interplay of relatedness and social structure also have implications for conservation-based studies. A review of recovery trends in cetaceans found that more social species may be particularly slow to recover from disturbance or exploitation at the population level (Wade, Reeves, & Mesnick, 2012). This may be due to the disruption of social networks, disturbances to which may interfere with reproductive activities or stop the flow of ecological knowledge (Snijders, Blumstein, Stanley, & Franks, 2017). Conservation of slow reproducing species should place stress on monitoring and improving conditions for reproduction as well as survival when assessing conservation risks (Manlik et al., 2016). This will require an understanding of the demographics and mating system of a population, which often can be uncovered only from high quality genetic relatedness data.

SNPs are set to become the marker of choice for relatedness estimation, and the advantage of SNPs over the more commonly used microsatellites have been reviewed extensively (Attard et al., 2018; Morin, Luikart, Wayne, & Palsbøll, 2004; Weinman, Solomon, & Rubenstein, 2015). SNP genotyping is still expensive relative to microsatellite analyses for many ecological and conservation-based projects (Flanagan & Jones, 2019; Puckett, 2017), but the increase in information provided may justify these costs depending on the research question. In our population at least, assigning a pair to a specific kinship category can require thousands of markers but only a couple dozen individuals, while inference about relatedness and social structure can be obtained from only a few hundred markers if a hundred individuals or more are available.

Our results both demonstrate feasibility and provide practical guidelines for minimum sample sizes and sequencing depth for future studies of genetic relatedness in wild populations, as well as thresholds for post hoc interpretation of previous analyses. We look forward to the increased incorporation of relatedness data and pedigree reconstruction derived from SNP data in models seeking to answer questions about social evolution.

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DATA ACCESSABILITY

Real and simulated genotypes and all variables included in the social model are archived on Dryad, https://doi.org/10.5061/dryad.rq2dg6m (Foroughirad, Levengood, Mann, & Frere, 2019).

ETHICS STATEMENT

Sampling was conducted under University of the Sunshine Coast Animal Ethics Committee approval, application number AN/S/15/35, and the Department of Biodiversity and Conservations permits #SF007418, #SF007975, #SF006897, #SF007457, #SF009311, #SF008076, #SF009876.

AUTHOR CONTRIBUTIONS

V.F. and C.F. designed research. V.F. and A.L. collected samples for genetic analysis and conducted lab work. J.M. collected and provided behavioural and demographic data. V.F. analyzed data and wrote first draft of the manuscript. All authors edited and approved the final version.

ORCID

Vivienne Foroughirad https://orcid.org/0000-0002-8656-7440
Alexis L. Levengood https://orcid.org/0000-0003-2729-5366
Céline H. Frère https://orcid.org/0000-0002-9671-2138

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